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THE EFFECT OF LOCAL APPLICATION OF Ca, K, AND Na ON THE TEMPERATURE CENTER STIMULATED BY VARIOUS PYROGENIC SUBSTANCES

O. Kym

Translation of "Die Beeinflussung des durch verschiedene fiebererzeugende Stoffe erregten Temperaturzentrums durch lokale Applikation von Ca, K und Na," Archiv für Experimentelle Pathologie und Pharmakologie, Vol. 176, 1934, pp. 408-424

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16. Abstract Intercerebral injection of $CaCl_2$ in rabbits prevented the fevers normally produced by intramuscular injection of β-tetrahydronaphthylamine, ergotoxin, or hay infusion. When administered in the course of a fever produced by one of these agents, $CaCl_2$ halted the temperature rise and soon returned temperature to normal levels. Neither NaCl nor KCl showed these effects. Intracerebral injection of $CaCl_2$ also caused disturbances of balance. Anatomical localization of the calcium injections in the tuber region indicated the presence there of certain thermoregulatory and static-regulation systems.

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THE EFFECT OF LOCAL APPLICATION OF Ca, K, AND Na ON THE TEMPERATURE CENTER STIMULATED BY VARIOUS PYROGENIC SUBSTANCES

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Demole [1] and Cloetta and Fischer [2] have demonstrated /408* the sedative or soporific effect of intracerebral Ca injections in the region of the infundibulum in cats, dogs, rabbits, and rats. Bunichi-Hasama [3] found that Ca injections in the tuber region of the normal rabbit regularly cause temperature decreases. The objective of the following work is to determine the effect of the endogenous cations Ca, K, and Na upon local application into the temperature center stimulated by pyrogenic substances. The experiments were conducted on rabbits. As fever agents[1], I employed intramuscular β-tetrahydronaphthylamine HCl (β-T-HCl), and intravenous ergotoxin and hay infusion. After repeated determination of the individual temperature curves for the animals concerned produced by a specific dose of one of these fever agents (cf. preliminary experiments, Figs. 1 through 7), the attempt was made in the principal experiment, for the animals first treated in this fashion, after preceding application of a fever agent, to determine the extent to which temperature regulation is affected by intracerebral injection of Ca, K, or Na, and whether there are any characteristic differences in the effects of the various cations. In the following, I will discuss a number of observations, beginning with the influence of intracerebral Ca application on the β-T fever.

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[1] [Translator's note: the German word translated "fever agent" actually means "antipyretic," but the context indicates that the author employs it to mean "pyrogen." This term will be translated as "fever agent" throughout.]

* Numbers in the margin indicates pagination in the foreign text.

I. Animal Experiments with β-T

1. The Effect of Intracerebral Ca Injection

As experimental animals I used in all experiments rabbits weighing 2 to 3 kg; smaller animals are not suitable for these experiments, since then intramuscular injection -- the optimum method for inducing fever -- is unreliable due to the smallness of the muscle. The injection can easily get into the subcutis, so that the fever effect is uncertain or absent -- this holds particularly for rabbits whose temperature regulation is inherent- /409
ly not very precise. For this reason, only experimental animals were used in which the temperature rise induced by the β-T was at least 1 to 1.5°C, and resulted in a fever of at least 40°. The administered quantity of β-T was, per 1000 g body weight, 0.7 to 0.8 cc of a 3% solution = 21 to 24 mg per kg. In a total of 28 β-T experiments, it caused a temperature elevation of 1.9° on the average; the maximum fever obtained was actually 3.2° above normal temperature. This fever agent is known to have a central effect [4]. Its effects were usually detected after 5 to 10 min in a temperature rise, motor and psychic excitation, acceleration of respiration and pulse, and in dilation of the pupils and protrusio bulbi. With striking consistency, all these manifestations reached their maximum after 75 min with a temperature rise to about 41°. Then there was a regular, slow temperature drop, returning the temperature to its initial value after about another 120 min. If the β-T dose was then increased to 1 cc and more of the 3% solution per kg, death through cardiac arrest was regularly observed after 75 to 90 min, preceded by hyperpyrexia and the most violent excitation. Since, for the injection experiments with cations, only those animals were used in which a β-T fever effect could be reliably anticipated, the /410
animals were repeatedly tested in preliminary experiments for their response to this fever agent, and only those animals used in the injection experiment which had repeatedly exhibited a

strong fever reaction. The same procedure was also employed with the other fever agents, so that a further discussion of the experimental technique will not be required at that point. In general, 8 days were allowed to elapse after the first positive preliminary experiment before the next one was performed, in order to be certain of the complete recovery of the animal. In the second preliminary experiment, the amount of the dose was modified on the basis of the effect obtained with a specific dose in the first experiment. In this way, the optimum individual β-T effect was found for each animal (cf. Fig. 1, preliminary experiment). After the second positive preliminary experiment, another 8-day wait was interpolated. Then the intracerebral calcium application into the base of the diencephalon was performed, with simultaneous intramuscular application of the optimum β-T dose found in the preliminary experiments.

Technique of Intracerebral Injection

Local anesthesia of scalp and periosteum in the region of the intersection of the sagittal and coronal sutures by means of 1 cc 0.5% novocaine solution. Exposure of periosteum in longitudinal section. Trepanation of cranium on both sides in the posterior angle formed by the sutures, 1.5 mm behind coronal suture and 1.5 mm laterally from sagittal suture. Diameter of trepanation aperture about 1.5 mm. Through it the rounded-off needle (0.4 mm thick) of the tuberculin syringe is inserted 15 to 16 mm deep perpendicular to the surface of the cranium. In this way, if the needle is guided correctly, the parainfundibular region 1 mm above the base of the brain near the median plane is reached. To the left and right of this point, injection of 0.05 cc of thedissolved cations in the form of their chlorides, where equimolar solutions were employed, i.e. 2.5% $CaCl_2$, 1.3% NaCl, and 1.7% KCl solutions. The path of the injection was stained by addition of Congo red, which provided an anatomical control of the localization of the injection without modifying the

Preliminary experiment = dotted lines. ↑: injection of 0.7 cc 3% β-T HCl solution per 1000 g = 1.61 cc, intramuscular. Ca experiment = solid line. ↓: intramuscular injection of 1.61 cc β-T HCl solution. ↡: intracerebral injection of 0.05 cc 2.5% $CaCl_2$ solution on both the left and right sides.

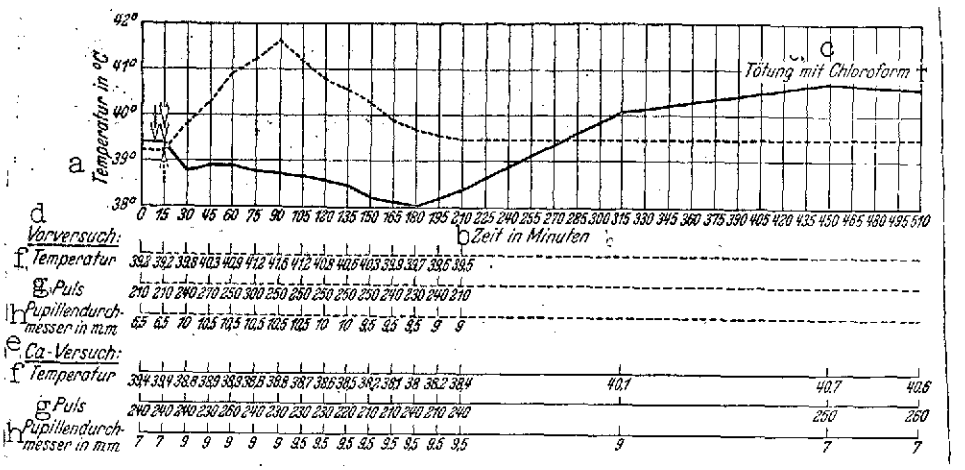

Fig. 1. Inhibition of β-T fever by simultaneous intracerebral $CaCl_2$ injection in the infundibular region of the diencephalon. In contrast to the preliminary experiments, temperature remains completely normal until the beginning of the puncture fever.

Key:
a. Temperature in °C
b. Time in min
c. Killing with chloroform
d. Preliminary experiment
e. Ca experiment
f. Temperature
g. Pulse
h. Pupil diameter in mm

cation effects, since Congo red itself has no effect, as Cloetta and Fischer [2] demonstrated. However, the solutions and the dye should not be mixed until immediately before the injection, in order to prevent blockage of the cannula due to flocculation. Immediately after the injection, which must be carried out rapidly, the head wound is again anesthetized with novocaine solution and closed with clamps.

The animals treated with intramuscular injection of β-T and simultaneous intracerebral Ca injection were observed for at least 9 hours. The general behavior of the animals, and particularly the changes in temperature, pulse and pupil width were checked /411 at small time intervals. The the animals were killed with chloroform. A postmortem examination was performed on the brain hardened in chloroform for verification and precise localization of the puncture or the injection site.

Whenever the injection localization was correct, the β-T experiments performed on 16 animals with intracerebral Ca injection consistently showed that there was never any temperature rise in spite of the intramuscular β-T injection, when calcium entered the tuber region on both sides.

From the series of 16 experiments, I have selected two of the most frequently observed patterns, depicted in Figs. 1 and 2. These two patterns differ that in the first case (Fig. 1), temperature remained normal after intracerebral Ca injection, while it became distinctly subnormal in the other case (Fig. 2). Hence, when injected intracerebrally in a total amount of about 1 mg Ca, the calcium sometimes appears to influence the temperature rise expected from the β-T in the sense of an overcompensation, which suggests that an inhibiting effect on the thermal tuber functions might be obtained with even smaller amounts of Ca. In the following we will sketch the reactions occurring with a certain regularity in animals upon intracerebral Ca injections. This symptomatic description should therefore command a certain neurological interest, because, suprisingly, a sequence of static disturbances was regularly found, which was /412 related to the localization of the Ca injection in each case. (See below.) This appears to us to be a not insignificant secondary finding, which is distinguished from other localization experiments in that the resulting symptoms were not brought about

Preliminary experiment = dotted lines. ↓: injection of 0.7 cc 3% β-T HCl solution per 1000 g = 1.45 cc, intramuscular. Ca experiment = solid lines. ↑: intramuscular injection of 1.45 cc β-T HCl solution. ↕: intracerebral injection of 0.05 cc 2.5% $CaCl_2$ solution on both left and right.

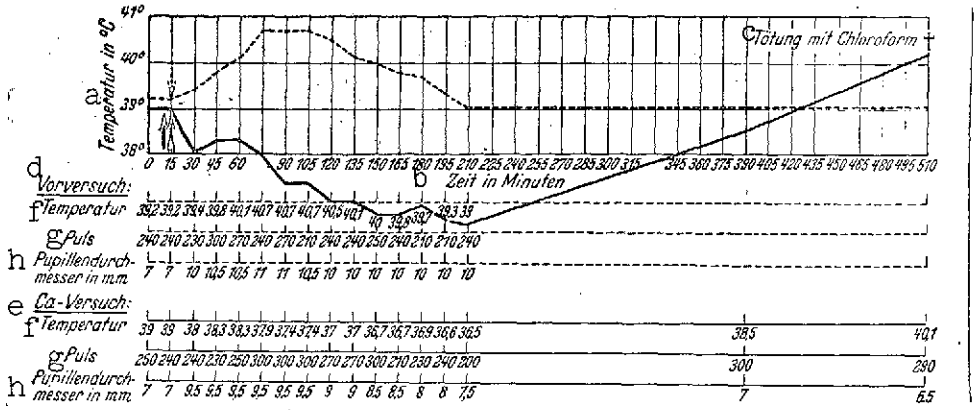

Fig. 2. Inhibition of β-T fever by simultaneous intracerebral $CaCl_2$ injection in the infundibular region of the diencephalon: instead of the pyretic β-T rise (preliminary experiment), the $CaCl_2$ injection caused a drop to a subnormal temperature.

Key:
a. Temperature in °C
b. Time in min
c. Killing with chloroform
d. Preliminary experiment
e. Ca experiment
f. Temperature
g. Pulse
h. Pupil diameter in mm

by mechanical injury, but by enrichment of an endogenous ion. In essence, the phenomena observed in connection with the Ca injections showed the following typical pattern:

first phase: Just after the intracerebral injection, the animal remains quiet for a few minutes, staggers to the left and right, and then unexpectedly tips over onto its side. It tries in vain to right itself, struggles violently with the forepaws, and rolls about its longitudinal axis. Should it accidentally

regain its normal posture, it runs forward and backward and then tips over again. Motor excitation, astasia, and ataxia predominate for 30 min. Temperature remains at 38.9°, i.e. normal.

second phase: The above ataxic and astatic symptoms are less distinct, and the excitation decreases. The animal remains on its side and moves very little. The excitation breaks through only sporadically, the animal -- generally lying on its side -- extending both rear paws spastically and paretically into the air and making violent running movements with the forepaws. However, it always relaxes after a few seconds. This second phase lasts about 15 min. Temperature remains at 38.9°.

third phase: Astasia and ataxia are largely relieved, and the animal can now maintain a sitting posture. It is quiet and somewhat somnolent, and the musculature is hypotonic. Reflexes are difficult to elicit. This recovery phase lasts about 2 hours. During this period as well, the animal is completely free of fever (temperature = 38°), i.e. at a time at which the β-T fever would have long since appeared without intracerebral Ca injection.

fourth phase: The behavior of the animal is completely normal. This phase usually begins about 210 min after the start of the experiment. In this phase as well, temperature remains at about 39°, i.e. completely normal.

fifth phase: = phase of the injection fever, appearing between 300-360 min after intracerebral injection, i.e. at the same time as observed without preceding β-T application. As a consequence of the injection fever, temperature rises to about 40.7°.

After the animal is killed with chloroform, the examination (example) shows: left end of puncture 0.3 mm laterally from the median plane and 1.2 mm vertically above the base of the brain,

precisely parainfundibular. Right: puncture end 1.5 mm laterally /413
from the median plane and 1.5 mm vertically above the base of the brain, also precisely parainfundibular. Both punctures are therefore well localized.

The main result of these and all other correspondingly successful Ca experiments is that intramuscular β-T injections causing temperature rises to as much as 41.6° have no effect when a certain minimum Ca amount simultaneously enters the tuber region. In my experiments, I used on each side 0.05 cc of a 2.5% $CaCl_2$ solution = 0.0025 g $CaCl_2$ total = about 0.001 g Ca.

The duration of the Ca effect, which we have to view as a type of blocking of the temperature regulating regions, could be approximately determined in the following manner: the β-T effect is not just delayed, but completely suppressed by the basal Ca injection. Since, with the employed dosage, the normal duration of the β-T effect until the fever and the other β-T symptoms disappear is 210 min on the average, the inhibiting effect of the calcium must last at least as long, i.e. 3 1/2 hours. On the other hand, since the injection fever begins, on the average, 300 min after the Ca injection, i.e. at the time repeatedly confirmed in other injection tests, and reaches the same level in the course of about 1 1/2 hours as in other injection fever experiments without Ca injection, it can be concluded that the effect of the Ca injection has disappeared by the beginning of the injection fever. Accordingly, the duration of the Ca effect would be at least 3 1/2 hours and no more than 5. Therefore, after at most 4 hours, the injected calcium should have been completely transported away from the tuber region so that the biochemical change in the state of the cells induced by the calcium will disappear.

In connection with the discussion of the injection fever, we should also point out that injection fever offers a further

possibility for checking the correct placement of the intracerebral injection. Since the injection fever is associated with the tuber region, its appearance can be viewed as physiological proof of the successful injection into the tuber. Thus, in addition to the anatomical findings, the occurrence of injection fever will demonstrate the correct localization of the intracerebral injections.

These experiments having shown that intracerebral injection of calcium can have a prophylactic antipyretic effect when the intramuscular application of β-T is carried out simultaneously, the objective of the following series of experiments was to determine whether the calcium would also have an effect after the fever reaction had already appeared, i.e. whether it was symptomatically antipyretic. For this purpose, we injected a number of animals with the customary dose of β-T and waited for fever symptoms to appear. Then Ca was applied intracerebrally, /414 whereupon the fever continued to rise for a short time (about 10 min). Then, however, the temperature dropped back to normal, a premature drop in comparison with the β-T preliminary experiment. Injection fever and postmortem examination proved the good placement of the calcium. The experiments therefore show that intracerebral calcium injection is also effective after the β-T fever has already appeared and that it retains its inhibiting action on the temperature-regulating functions of the tuber region even when the latter has already been stimulated by the pyretic action of β-T.

What can we conclude from these Ca experiments? The important theoretical question in the interpretation of my experiments is doubtlessly the following: do these intracerebral Ca injections produce a specific Ca ion effect or can analogous changes in the function of basal centers be induced by other ions as well? Is the effect of the injections perhaps only a nonspecific osmotic one or is it perhaps a specific ionic one?

In order to reply to these questions, I undertook corresponding experiments with intracerebral injections of Na, which will be very briefly discussed in the following.

2. Effect of Intracerebral Sodium Injection

If an animal received simultaneous applications of intramuscular β-T-HCl in the customary dose and NaCl in the stated amount in the tuber region, a fever curve resulted which was identical with the clinical picture of the β-T effect, exactly as if no intracerebral injection had been made. The first consequence of the intracerebral injection was the subsequent injection fever appearing after the usual latency time of 3-4 hours, after the β-T fever had already disappeared. This experiments shows that the operative treatment itself was irrelevant, i.e. it did not prevent temperature regulation. If, in another experiment, after β-T had been injected, and after NaCl had been injected and proved ineffective, the temperature rise and all known β-T symptoms having appeared, a Ca injection was now made in the same region of the brain, temperature immediately ceased rising and quickly returned to normal. Sometimes even subnormal temperatures were observed. These experiments clearly show that the inhibition of tuber functions by calcium is a quite specific ionic effect, corresponding to the hypnotic effect of calcium in intracerebral injection into the infundibular region. In contrast, the injection of NaCl into the tuber region had no detectable ion effect at all, but only the nonspecific one of the mechanical injection fever stimulus, which can be produced in the same manner by Ca, Na, or just by the puncture itself. This is proved by experiments on normal animals, in which injection fever could be induced both by $CaCl_2$ and NaCl injections into the tuber region; the latency time was the same for both injected substances. This is also another proof that the specific Ca effect on the fever center has disappeared at the point when the injection fever starts, since otherwise the injection fever induced by Ca

/415

injection would have to occur later than that produced by Na injection.

The experiments with intracerebral Na injection therefore show that Na, in opposition to Ca, when injected into the tuber region, exerts no specific influence on the temperature-regulating functions of this region. This finding is consistent with correspondingly negative results from application of NaCl into the infundibular region.

3. Effect of Intracerebral K Injection

The experiments with intracerebral K injections must be judged with some caution, since the effects were much less constant and regular than in the Ca experiments. In the end, two types of K effects were found: in one, there was after the K injection a slight temperature drop lasting for a short time (about 15 min) which was followed by a temperature rise, far exceeding that of the β-T preliminary experiment. (Cf. Fig. 3). It thus appears as if potassium favors the pyretic action of β-T, which probably implies that the potassium exerts a sensitizing /416
influence on the temperature-regulating tuber region, promoting the tendency towards hyperpyrexia. Unfortunately, the K effects were not as clear in all experiments as in the case described in Fig. 2. In the majority of experiments, the stimulating influence on the temperature center was not this clear: in this second type, there was no primary temperature drop, while the stimulating effect on the temperature center was much less distinct and the fever curve did not exceed that of the normal β-T effect. On the other hand, potassium appeared to prolong the β-T effect in the sense that the temperature drop after the maximum of the β-T effect took place more slowly, so that the β-T effect could frequently not be separated from the injection fever now beginning. In this type of K effect as well, the correct localization of the K injections could be confirmed by anatomical verification. In

all cases, the K experiments permit the conclusion that potassium, injected into the tuber region, cannot suppress the β-T fever, and that a stimulating effect on the temperature center can be detected in individual cases.

Preliminary experiment = dotted lines. ↓: injection of 0.8 cc 3% β-T HCl solution per 1000 g = 1.5 cc, intramuscular. K experiment = solid lines. ↑: intramuscular injection of 1.5 cc β-T HCl solution. ↕: intracerebral injection of 0.05 cc 1.8% KCl solution on left and right.

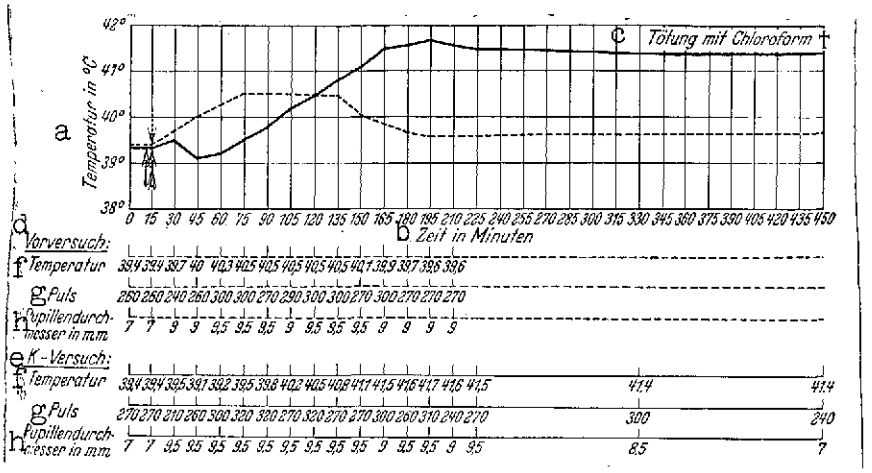

Fig. 3. Intensification of β-T fever by simultaneous intracerebral K injection into the infundibular region of the diencephalon: the temperature drop is moreover much delayed in comparison with the preliminary experiment.

Key:
a. Temperature in °C
b. Time in min
c. Killing with chloroform
d. Preliminary experiment
e. K experiment
f. Temperature
g. Pulse
h. Pupil diameter in mm

II. Animal Experiments with Ergotoxin and Intracerebral Ca Injection

We employed ergotoxin phosphate from Borroughs and Wellcome in 5% aqueous solution. Its poor solubility caused some

difficulties. In addition, the animals differed greatly in their response to ergotoxin, in the sense of a fever reaction. The majority of animals remained free of fever, some even showing subnormal temperatures, while fever was found in only a small number. In this case, the applied ergotoxin dosage played only a subordinate role, since fever could not be produced in ergotoxin-refractory animals either by increasing or reducing the dose, while ergotoxin-sensitive animals often showed consistent temperatures rises with quite different dosages. As a rule, I employed the high dosage of 1 mg ergotoxin per 1000 g body weight, which was tolerated by all but one animal. Averaged over the seven positive experiments, it produced a temperature rise of 1.4°, and as much as 2° in one animal. This variable behavior of the animals means that the repeated preliminary experiment was particularly important here, since an individual ergotoxin sensitivity had to be determined first for each animal. Even with this cautious procedure, the fever effect was still somewhat variable. Fevers sometimes appeared within 30 min, but sometimes not until 140 min after the intravenous injection. Its duration likewise varied significantly. Spontaneous defervescence often appeared after 5 hours in a preliminary experiment, but sometimes not for 12 hours. In contrast to β-T, ergotoxin induced a distinct, prolonged fever. This fact is noteworthy for evaluating the calcium effect, since the prolonged ergotoxin fever can still be in progress at the time when the injection fever should start, so that no boundary can be drawn between the two types of fevers, since they are chronologically and clinically (in accordance with special features of fever symptomatology) inseparable. Therefore, functional verification of injection localization, made possible by the appearance of injection fever in the β-T experiments, is now precluded, so that only postmortem findings can be utilized as controls on localization. There are also considerable symptomatic differences between the ergotoxin fever and the β-T fever. In contrast to the β-T fever, the animals were strikingly quiet during the ergotoxin effect, sat on the same spot, and showed, relative

to the height of the fever, a slow pulse (pronounced bradycardia) and narrow pupils, which, in addition to the regularly appearing diarrhea, emphasized the vagotonic character of the ergotoxin side effects. In this respect, ergotoxin is a counterpart to the distinctly sympathicotropic β-T (cf. Fig. 4).

Preliminary experiment = dotted lines. ⋮↓: injection of 1.2 mg ergotoxin per 1000 g = 5.6 cc 5% solution, intravenous. Ca experiment = solid lines. ↑: intravenous injection of 5.6 cc ergotoxin solution. ↕: intracerebral injection of 0.05 cc 2.5% $CaCl_2$ solution on left and right.

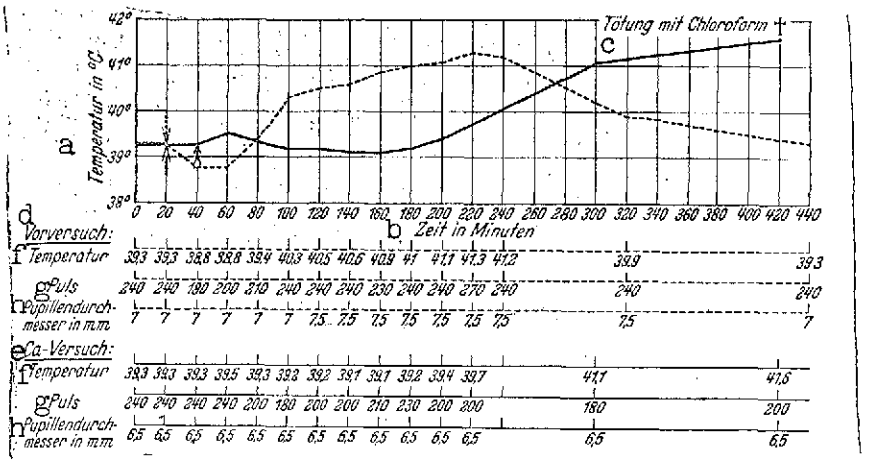

Fig. 4. Inhibition of ergotoxin fever by intracerebral $CaCl_2$ injection in the infundibular region of the diencephalon. In opposition to the preliminary experiment, temperature remains normal until the beginning of the injection fever.

Key:
a. Temperature in °C
b. Time in min
c. Killing with chloroform
d. Preliminary experiment
e. Ca experiment
f. Temperature
g. Pulse
h. Pupil diameter in mm

The experiments with intracerebral Ca injections and simultaneous intraveous administration of ergotoxin resulted in static disturbances similar to those in the β-T experiments.

However, the main result is that the Ca injections regularly suppress the expected temperature rise (cf. Fig. 4), and even resulted in distinctly subnormal temperatures in individual cases (cf. Fig. 5). In order to organize the experiments to bring out /418
even more clearly the inhibiting action of calcium, on the day following the ergotoxin calcium test in some of these experiments, the now fever-free animal received once again the same ergotoxin dose as on the day before. As in the preliminary experiments, a marked fever rise was observed, which clearly proved that the animal would have had a fever on the day before even without the calcium injection, and had not become ergotoxin-insensitive in the interim. These experiments, like the β-T experiment before them, also showed that Ca injection induces only a temporary disturbance of the functions of the temperature center and not a permanent one. From this we can perhaps conclude that Ca also intervenes physiologically as a functionally inhibiting ion (as on the "waking center") in central temperature regulation.

The fact that suppression of ergotoxin fever by intracerebral Ca injection is a specific Ca effect follows from the fact that NaCl injection into the tuber region does not suppress the ergotoxin fever any more than it did the β-T fever.

III. Experiments with Intracerebral Ca Injections After Hay Infusion Application

We gave intravenous injections of 0.7-0.8 cc of our very effective pyretic hay infusion per 1000 g body weight. The temperature rise always began very rapidly and reliably after 15 min. It was an average of 1.7° in six experiments, with a /419
maximum of 2.3°C. In spite of the high temperature, the animals remained very quiet. The duration of the fever fluctuated. The slow decline in fever usually did not begin for 3 1/2 to 4 hours, so that the temperature drop was still slight at the time when the injection fever began. Thus, as in the ergotoxin experiments, the

Preliminary experiment = dotted lines. ⇣: injection of 1 mg ergotoxin per 1000 g = 3.5 cc 5% solution, intravenous. Ca experiment = solid lines. ↑: intravenous injection of 3.5 cc ergotoxin solution. ↕: intracerebral injection of 0.05 cc 2.5% $CaCl_2$ solution on left and right.

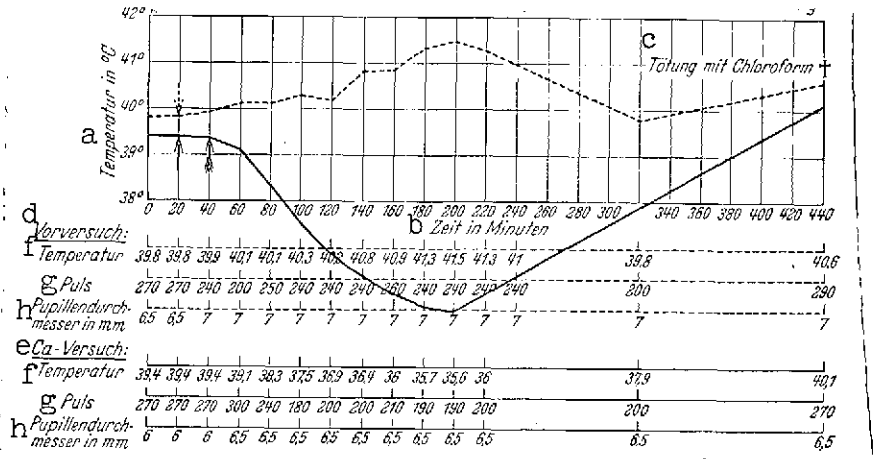

Fig. 5. Inhibition of ergotoxin fever by intracerebral $CaCl_2$ injection into the infundibular region of the diencephalon. Instead of the pyretic ergotoxin rise (preliminary experiment), $CaCl_2$ injection causes a drop to subnormal temperature.

Key:
a. Temperature in °C
b. Time in min
c. Killing with chloroform
d. Preliminary experiment
e. Ca experiment
f. Temperature
g. Pulse
h. Pupil diameter in mm

injection fever resulting from the intracerebral injection could not be separated from the prolonged hay infusion fever, so that here too, only postmortem findings could provide information on the injection localization. In order to avoid any aftereffects of the hay infusion (so-called cumulative infusion effect) in these experiments, the two preliminary experiments and the Ca experiment were conducted with 8-day intervals between them. In response to the intravenous hay infusion injection, all seven

animals showed very intense fever (see Fig. 6, preliminary experiments). It was now of particular interest to determine the effect of intracerebral Ca injection on this hay infusion fever, which had arisen on a completely different basis, and whose cause was certainly not just a central one. It turned out that the intracerebral application of calcium simultaneous with the hay infusion was able to completely prevent the onset of fever in all experiments. In most of the cases, there was a drop to subnormal temperatures (cf. Figs. 6 and 7). Having thus established the prophylactic antipyretic action of calcium, we conducted several other experiments showing that intracerebral injection of calcium also had an antipyretic effect after the hay infusion fever had already begun. The injected calcium not only prevented any /420
further temperature rise, it also hastened the return to normal temperature. Analogous experiments with intracerebral NaCl injections, which had not the slightest effect on the hay infusion fever, showed that this was a specific Ca effect in this case as well.

As a secondary finding, the previously described static disturbances were also observed in these experiments after the Ca injection. However, it was striking that all hay infusion animals, and only these, had their general well-being severely impaired by the Ca injection. During the entire duration of the Ca effect, the animals lay on their sides in a comatose state, and only rarely showed injection fever or continuing hay infusion fever, while the latter had regularly persisted into the injection fever in the preliminary experiments. The poor condition was in particularly striking contrast to the behavior of the same animals during the preliminary experiments, in which their condition was only slightly different from that of normal animals. As a cause of this comatose state, I suggested cerebral hemorrhages caused by the Ca injection, but this was never the case. The injection viewed as an operation, i.e. as an injury, could not be held

Preliminary experiment = dotted lines. ↕: injection of 0.7 cc hay infusion per 1000 g = 1.68 cc, intravenous. Ca experiment = solid lines. ↓: intravenous injection of 1.68 cc hay infusion. ↧: intracerebral injection of 0.05 cc 2.5% $CaCl_2$ solution on left and right.

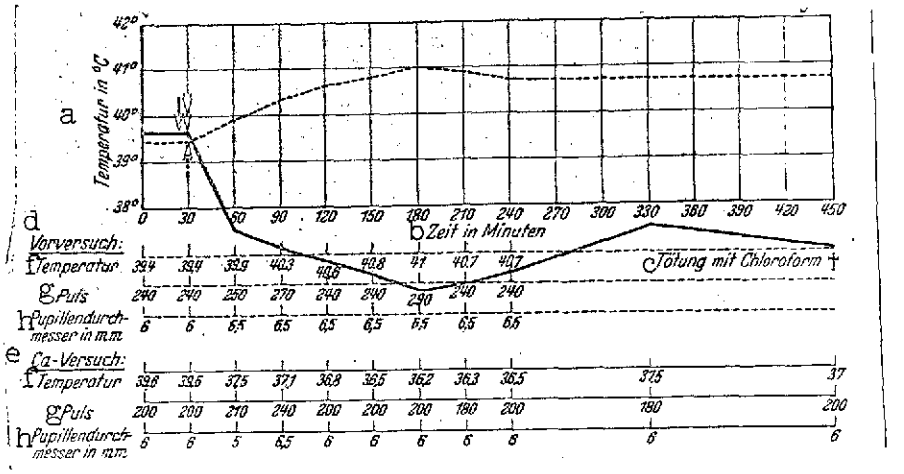

Fig. 6. Inhibition of hay infusion fever by simultaneous intracerebral $CaCl_2$ injection in the infundibular region of the diencephalon. Instead of the pyretic rise of long duration (preliminary experiment), there was a drop to subnormal temperature. Later, slight rise in normal temperature (instead of injection fever).

Key:
a. Temperature in °C
b. Time in min
c. Killing with chloroform
d. Preliminary experiment
e. Ca experiment
f. Temperature
g. Pulse
h. Pupil diameter in mm

responsible for this, because otherwise, the β-T, ergotoxin, and NaCl experiments with the same operation would have had to yield the same data. Theoretically, there is now the possibility that calcium causes collapse in hay infusion fever, but the most probable explanation is that the collapse comes about because the /421
Ca injection deprives the organism of its "fever capacity" and this does so much harm to the animal that injection fever is absent due to general exhaustion. The process can thus be

Preliminary experiment = dotted lines. ↑: injection of 0.7 cc hay infusion per 1000 g = 1.6 cc, intravenous. Ca experiment = solid lines. ↓: intravenous injection of 1.6 cc hay infusion. ↓: intracerebral injection of 0.05 cc 2.5% $CaCl_2$ solution on left and right.

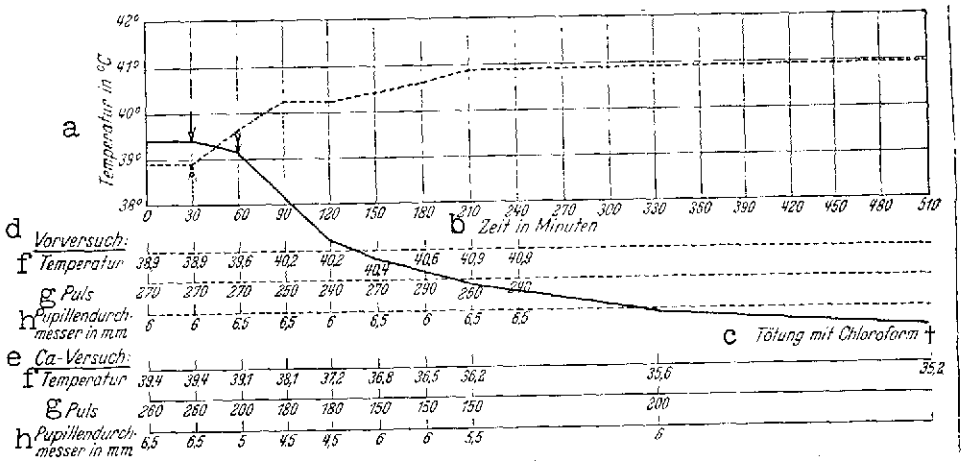

Fig. 7. Inhibition of hay infusion fever by simultaneous intracerebral $CaCl_2$ injection in the infundibular region of the diencephalon. Temperature shows continuous drop to subnormal temperature, persisting during the period of the injection fever.

Key:
a. Temperature in °C
b. Time in min
c. Killing with chloroform
d. Preliminary experiment
e. Ca experiment
f. Temperature
g. Pulse
h. Pupil diameter in mm

imagined as follows: the fever masks the collapse induced by the hay infusion, but the collapse must immediately manifest itself when, for some reason, the fever does not appear.

IV. Anatomical Localization of Injection Sites in Tuber Region and Demarkation of Temperature Centers

The simultaneous projection of the localizations of all Ca injection sites into the plane of the base of the diencephalon

(cf. Fig. 8) comprises a region whose limitation implies that Ca is only effective within the diencephalon zone described by Isenschmid and Schnitzler [5] as the temperature center. Frontally, this begins precisely at the posterior boundary of the chiasm, then includes the retrochiasmal, the parainfundibular, and the superjacent suprainfundibular regions, extending to about 2.5 mm vertically above the base of the brain, in accordance with my experiments on rabbits. The retroinfundibular region, including the corpora mamillaria, is also part of this zone. Calcium injections outside this region did not inhibit the temperature-regulating function. As Fig. 8 shows, the area of the region producing the injection fever is somewhat larger than that zone within which thermal regulation can be inhibited by Ca injection. Interestingly, the Ca injection likewise had no effect, if only one of the punctures lay outside the circumscribed region; thus, bilateral Ca injection is required for functional elimination of the thermoregulatory zone. Calcium injected into the cerebral cortex as a control proved to have no effect whatsoever.

V. Analysis of Static Disturbances /422

The static disturbances resulting from basal Ca injection may be of special interest inasmuch as Bechterew [6] described similar observations in his cerebral injury experiments on dogs. The astatic and ataxic manifestations I found in rabbits agree very well with his findings. However, in my injection experiments, I never found any such eye symptoms (deviation of the bulbus, etc.), although he made his incisions at roughly the same point as my injections. The static disorders which I observed are, by and large, consistent with those known from cerebellum injuries. For this reason, the cerebella of my rabbits were examined for any injuries, but always with negative results, so that the direct cerebellum due to Ca injections can be ruled out. Therefore, it may be assumed that not only the

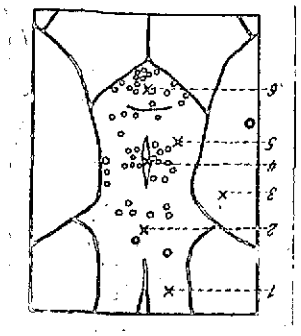

Fig. 8. Schematic representation of Ca injection sites by projection onto basal surface of diencephalon. ● = Ca injections preventing β-T, ergotoxin, and hay infusion fevers but causing injection fever. o = Ca injections having no effect on β-T, ergotoxin, and hay infusion fevers, but still causing injection fever. (cf. text). 1 = nervus opticus; 2 = chiasm; 3 = basal edge of temporal lobe; 4 = infundibulum; 5 = tuber region; 6 = corpora mamillaria.

cerebellum, but also the diencephalon is of functional importance in the maintenance of balance. On the basis of his experiments with injuries localized at the base of the diencephalon, Bechterew believes the wall of the third ventricle to be the site of static centers. It is also conceivable that the statically sensitive diencephalon region has a regulatory influence on the tonus and the reactivity of the static cerebellum centers in the sense of a regulatory arrangement coordinates with the cerebellum or superior to the latter. It should be emphasized that the static disturbances I induced are not to be considered as injury effects, but should be attributed to the action of the locally injected calcium. These effects appeared only after calcium injection, but never after sodium injection. Hence, this is probably a specific effect of the calcium ion. It is therefore not the operation, but the injected cation which is crucial for the disturbance of balance -- the purely mechanical effect of the injection is quite insignificant.

More detailed examination of the anatomical localization of the injections with static effects now showed that suprachiasmal Ca injection had no influence in the sense of astasia and ataxia, while retrochiasmal Ca injections produced by far the severest and longest-lasting disturbances of balance. Ca injections localized somewhat farther back, i.e. retroinfundibularly

and in the corpus mamillare showed less pronounced static disturbances, which were also of shorter duration than those with retrochiasmal Ca application. The following table shows the difference in duration of disturbances of equilibrium in relation to their localization somewhat more clearly; only injection sites with approximately symmetrical localization are included.

TABLE 1. DEPENDENCE OF INTENSITY AND DURATION OF STATIC DISTURBANCES ON LOCALIZATION OF Ca INJECTIONS IN DIENCEPHALON

Localization of Ca injections				
Localization	retrochiasmal	parainfundibular	retroinfundibular	Corpus mamillare
Duration of static disturbances in min	230	120	110	60
	175	90	80	60
	200	170	140	80
		135	160	80
		75	75	45
		75		30
Average	200	120	110	60

From Table 1 we obtain the interesting finding that the /423
duration and intensity of disturbances of balance caused by Ca decrease in a ratio of about 4:2:1 from the anterior tuber region (retrochiasmal) through the middle (para- and retroinfundibular) to the posterior (corpora mamillaria). The statically most sensitive areas are thus, in agreement with Bechterew, in the anterior half of the base of the diencephalon. The question of whether these are actually static "centers" is of quite secondary importance. In any case, the static cerebellum apparatus appears to be somehow functionally subordinate to this part of the brain, since blocking it alone is sufficient to cancel all regulation of balance. Biochemically, this ought to involve the same processes for the cells concerned, in the sense of a reduction of their specific function and those already demonstrated for the waking center [7] and in preceding experiments for the fever center.

Summary

1. Injection of 2.5% $CaCl_2$ solution in the amount of 0.05 cc on each side into the base of the diencephalon of rabbits blocks the onset of β-T, ergotoxin, and hay infusion fevers. This effect must be viewed as a specific effect of the calcium ion, which cannot be produced by basal injections of either potassium or sodium.

2. In analogous fashion, calcium injected on both sides into the temperature center can prevent any further temperature rise in already existing β-T, ergotoxin, and hay infusion fevers, and force a rapid return to normal temperature. Calcium therefore has an antipyretic effect which is both prophylactic and symptomatic, in that the functions of the thermoregulatory systems of the diencephalon can be reversibly inhibited in the sense of a blocking effect. /424

3. In addition to the antipyretic effect, basal injection of calcium also produces with equal regularity static disturbances, which likewise seem to be specific for calcium. These reversible disturbances of balance, which are not consequences of injury, indicate that in the tuber region, and in fact predominantly in the anterior half of the diencephalon, there are certain static regulatory systems which have a regulatory effect on the function of the static cerebellum centers.

4. Basal NaCl injections (0.05 cc on both sides, 2% solution) have no inhibiting effect on the onset, height, and duration of β-T, ergotoxin, and hay infusion fevers in simultaneous application, and no effect on the course of the fevers after they have already begun.

5. Basal injections of 0.05 cc of a 1.8% solution on both sides caused hyperpyrexia in some of the experiments after brief

inhibition of the β-T fever rise. In other experiments, the K injections did not affect the level of the β-T fever, but protracted the deline of the β-T fever curve. Hence, basal K injection in the tuber region has in a certain sense the opposite effect of the corresponding Ca application.

6. Anatomical localization of the Ca injections resulting in abolition of the thermoregulation of the diencephalon is consistent with the region of the so-called temperature-regulating centers found by Isenschmid and Schnitzler by other methods. Outside this region, Ca injections had no effect.

REFERENCES

1. Demole, V., Arch. f. Exper. Path. 120, 229 (1927).

2. Cloetta, M. and Fischer, H., Ibid. 158, 225 (1930).

3. Bunichi-Hasama, Ibid. 153, 291 (1930).

4. Cloetta, M. and Waser, H., Ibid. 75, 406 (1914).

5. Isenschmid and Schnitzler, Ibid. 76, 202 (1914).

6. Bechterew, W. C., Die Funktion der Nervenzentra, [Function of Nerve Centra], Vol. 2, Jena, 1909, p. 774.

7. Cloetta, M., Fischer, H., and van der Loeff, M. R., Archiv f. Exper. Path. 174, 589 (1934).